Spectroscopic studies on the oxygen-binding site of hemocyanins.

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Hemocyanins are multisubunit proteins which occur freely dissolved in the hemolymph of many molluscs (such as snails and squids) and arthropods (such as crabs, lobsters, spiders and scorpions). The ligand binding site of these non-heme respiratory proteins has long been of interest and is considered to contain two copper ions which reversibly bind 1 O_2 molecule. The structure and properties of hemocyanins have been reviewed frequently, most recently by Van Holde and Van Bruggen[1], Lontie and Witters[2], Lontie and Vanquickenborne[3] and Williams[4] and Lontie[5].

In the past decade hemocyanin research at the University of Groningen has developed into a joint project of the biochemistry, physical chemistry and structural chemistry departments. Both arthropodal and molluscan hemocyanins were studied by Konings[6], Dijk[7], Schoot Uiterkamp[8], Siezen[9], Van Driel[10], Brouwer[11], Schepman[12] and Kuiper[13]. The research included subjects as amino-acid sequence determination, examination of the quaternary structure, the structure and function relationship, and the active site. The present state of research on hemocyanin is excellently described in the Proceedings of the Vth Hemocyanin Workshop in 1976[14]. Our present knowledge of the active sites of hemocyanins is mainly based on spectroscopical techniques, because until now no high resolution X-ray structure of this protein is available. In this thesis we will describe spectroscopical measurements on hemocyanin and its modified state, with circular dichroism (CD), electron paramagnetic resonance (EPR), infrared (IR), magnetic susceptibility, resonance Raman and X-ray photoelectron spectroscopical (XPS) techniques and try to relate the results of these measurements with the structure of the oxygen-binding site. Specific introductions in each of the following five chapters will supplement this general introduction.

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